Pacific Science (1982), vol. 36, no. 3

A Note on the Structural Organization of the Cardiac Myofiber in *Nautilus pompilius*[1]

JAMES A. DYKENS,[2] CHARLOTTE P. MANGUM,[3] and JOHN M. ARNOLD[4]

ABSTRACT: The ultrastructure of the cardiac myofiber in *Nautilus* resembles that of bivalves more than the decapod cephalopods. The fiber is nonstriated, the mitochondrial density is relatively small and the cristae poorly developed, and the sarcoplasmic tubule system is either sparse or absent. These features suggest that the *Nautilus* heart is not highly adapted to enhance the transport of large volumes of oxygen to the tissues and that the adaptations found in the decapods arose within the class Cephalopoda.

THE VARIOUS CLASSES OF MOLLUSKS represent extremes of metabolic, locomotor, and cardiovascular performance. In most of the sedentary bivalves, for example, the oxygen transport and cardiovascular systems are not adapted to supply metabolizing tissue with large volumes of oxygen, and the capacity for anaerobiosis is highly developed. In the fast-swimming squids, the capacity for anaerobiosis is essentially nonexistent, and the performance of the oxygen transport and cardiovascular systems is truly remarkable.

A key component in the total dependence on oxygen is the reorganization of the cardiac myofiber to permit contraction frequencies of a magnitude unequalled elsewhere in the animal kingdom (Dykens and Mangum 1979). The nonstriated fiber of the bivalves is replaced in the cephalopods by an obliquely striated fiber that contains an extraordinary density of mitochondria with many more and much longer cristae. The mean fiber diameter, and thus the blood–mitochondrion diffusion distance, is smaller and the sarcoplasmic reticular system much denser in the cephalopods than in the bivalves. This adaptation reduces the distance over which Ca^{2+} must diffuse from the activation site to the site of sequestering during relaxation, and thus contributes to a small refractory period.

Hochachka, French, and Meredith (1978) examined the organization of intermediary metabolism in relation to the structural organization of four different muscles in *Nautilus pompilius*, including the heart. Since their micrographs were prepared for a different purpose, several of the features critical to the performance capability of the heart remain unclear. The question is particularly interesting since, in a number of physiological respects, *Nautilus* occupies an intermediate position between the bivalves and the decapod cephalopods, viz. cardiovascular performance, locomotor performance, the rate of total oxidative metabolism, and the capacity for anaerobiosis (Bourne, Redmond, and Johansen 1978; Hochachka et al. 1978; Johansen, Redmond, and Bourne 1978; Redmond, Bourne, and Johansen 1978).

We have reexamined the ultrastructure of the cardiac myofiber in order to compare it with the very different fibers found in bivalves and in decapod cephalopods.

METHODS AND MATERIALS

The ventricle was excised and fixed for 1 hr in 2.6% gluteraldehyde in seawater (osmo-

[1] This research was conducted as part of the *Alpha Helix* Cephalopod Expedition to the Republic of the Philippines, supported by National Science Foundation grant PCM 77–16269 to J. Arnold.

[2] University of Maine, Department of Zoology, Orono, Maine 04469.

[3] College of William and Mary, Department of Biology, Williamsburg, Virginia 23185.

[4] Pacific Biomedical Research Center, University of Hawaii, Kewalo Marine Laboratory, Honolulu, Hawaii 96813.

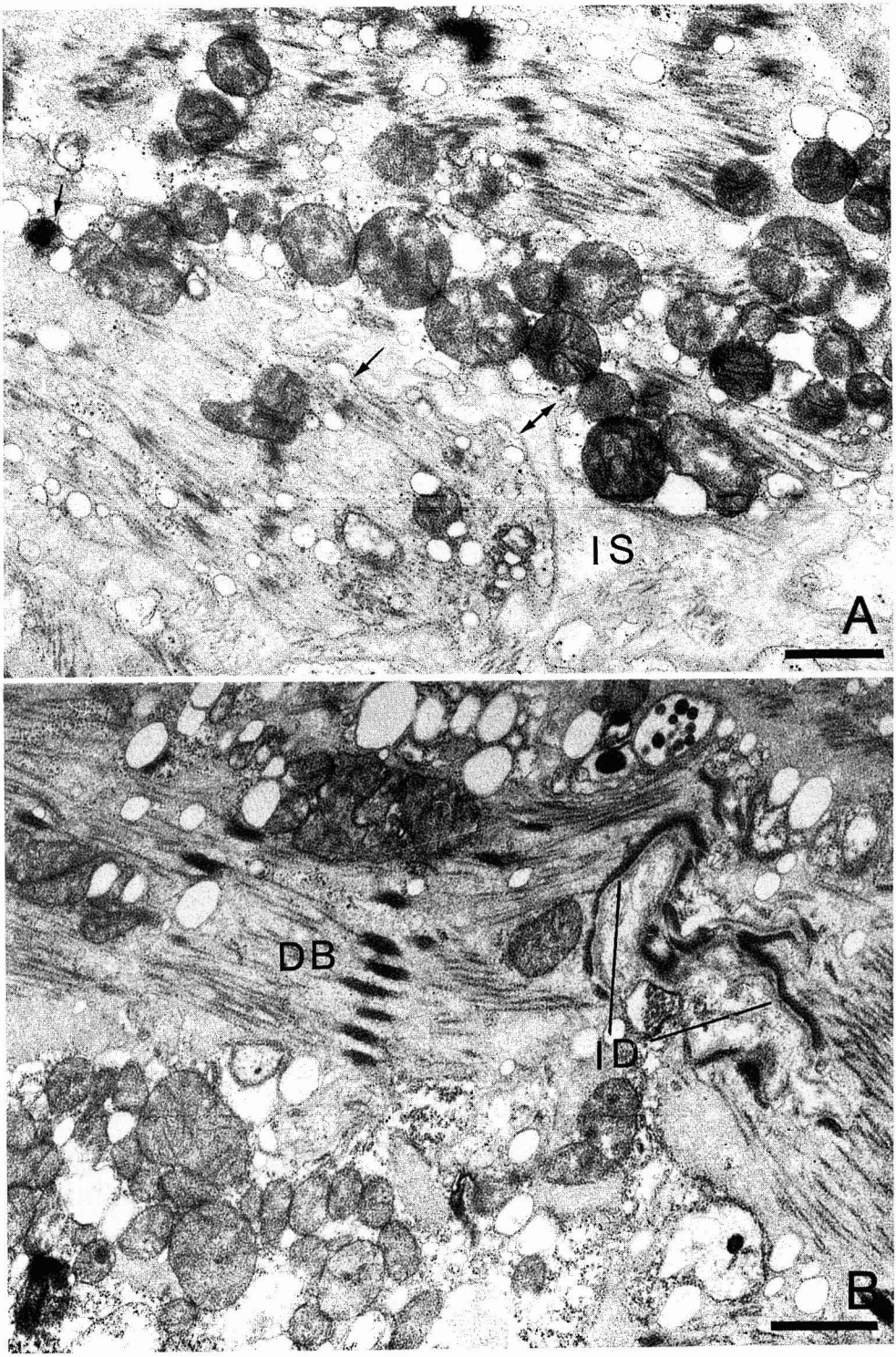

FIGURE 1. Transmission electron micrographs of *Nautilus pompilius* myocardium. *A*, intercellular space (IS) surrounded by serpentine sarcolemma (arrows), note number of open areas. *B*, cross section of intercalated disk (ID) and longitudinal section of dense bodies (DB). Bar indicates 1 μm.

lality 1003 mOsm, pH 7.2). Following a 1-hr rinse in filtered seawater, the tissue was post-fixed in 1% OsO_4 (pH 7.0). The tissue was embedded in Epon 812, cured at 60°C, sectioned, and stained with lead citrate (0.3% for 30 sec). The sections were examined with Zeiss 9S-2 and Phillips EM-201 electron microscopes.

RESULTS AND DISCUSSION

The *Nautilus* myocardium more closely resembles the bivalve myocardium than that of decapod cephalopods. As in the bivalves (Hayes and Kelly 1969; Irisawa, Irisawa, and Shigeto 1973; Rutherford 1972), the ventricle is composed of loosely organized molluscan nonstriated muscle with dense, or Z, bodies scattered throughout the sarcoplasm (Figure 1). In contrast, the decapod cardiac myofiber is obliquely striated, with Z bodies stacked linearly (Dykens and Mangum 1979, Jensen and Tjønneland 1977, Schipp and Schäfer 1969). The dense bodies are slightly larger (0.4–0.6 μm) in *Nautilus* than in the bivalve *Mercenaria mercenaria* (0.08–0.12 μm), and they do not form the attachment plaques seen in both *M. mercenaria* (Hayes and Kelly 1969) and the decapod *Rossia macrosoma* (Jensen and Tjønneland 1977). As in the bivalves, but not in the decapods, the myofilaments are oriented in several directions within the same myofiber and often run perpendicular to those of adjacent cells.

The cross-sectional area of *Nautilus* myofibers averages 35 μm^2 but ranges from 25 to 55 μm^2, which is somewhat larger than the squid fiber (Dykens and Mangum 1979). The cells are irregular in shape, with anastomotic processes frequently meandering through several planes of section. As in the bivalves, the sarcolemma is serpentine and difficult to trace. An extracellular space (0.2–0.8 μm) separates neighboring cells, but the extensive network of collagen fibers found in some bivalves (Hayes and Kelly 1969) is not seen. Sites of cellular attachment closely resembling the intercalated disks found in some decapods (Jensen and Tjønneland 1977, Schipp and Schäfer 1969) occur in *Nautilus* (Figures 1, 2). These structures do not occur in *Mercenaria mercenaria* and are also absent in the squid *Lolliguncula brevis* (Dykens and Mangum 1979).

The myofilaments account for 60–80% of the cross-sectional area of the myofiber and mitochondria for 10–30%. A similar ratio is found in bivalves, where only 5–50% of the area is occupied by mitochondria. On the other hand, both the cardiac myofiber and the mantle myofiber in squids tend to have a larger ratio of mitochondria to myofilaments, and mitochondria can occupy up to 80% of the cross-sectional area (Dykens and Mangum 1979, Moon and Hulbert 1975). In the decapods, the mitochondrial cristae are larger (0.8–1.2 μm diameter), more numerous, and longer than in the bivalves (Dykens and Mangum 1979, Jensen and Tjønneland 1977, Schipp and Schäfer 1969). The *Nautilus* mitochondria are smaller (0.6–0.9 μm diameter), and they usually contain less than ten poorly developed cristae, features that also resemble bivalves. As in most mollusks, although the mitochondria are distributed throughout the myofiber, they typically occur in groups that tend to concentrate in the core of the cell or directly beneath the sarcolemma (Figure 2).

As reported earlier (Hochachka et al. 1978), the tissue is characterized by numerous open areas that resemble vacuoles. These spaces range in diameter from 0.1 to 0.5 μm, and they occur most densely in the mitochondrial clusters. Partially disrupted mitochondria were observed in several of these "vacuoles," suggesting that they are artifacts of unknown origin formed during fixation. The normal appearance of other structures, however, such as the cell nuclei, Golgi apparatus, and endoplasmic reticulum, suggests that the vacuoles may not be due to osmotic imbalance. Their presence complicates the identification of a sarcoplasmic reticular system. Tubulelike structures with patent lumina of 0.01–0.10 μm diameter are scattered throughout the myofilaments (Figure 1). In both Figures 1 and 2 and in the micrographs of the cardiac myofiber shown by Hochachka et al. (1978), these tubulelike structures could represent a tangential section of a round vacuole, made at

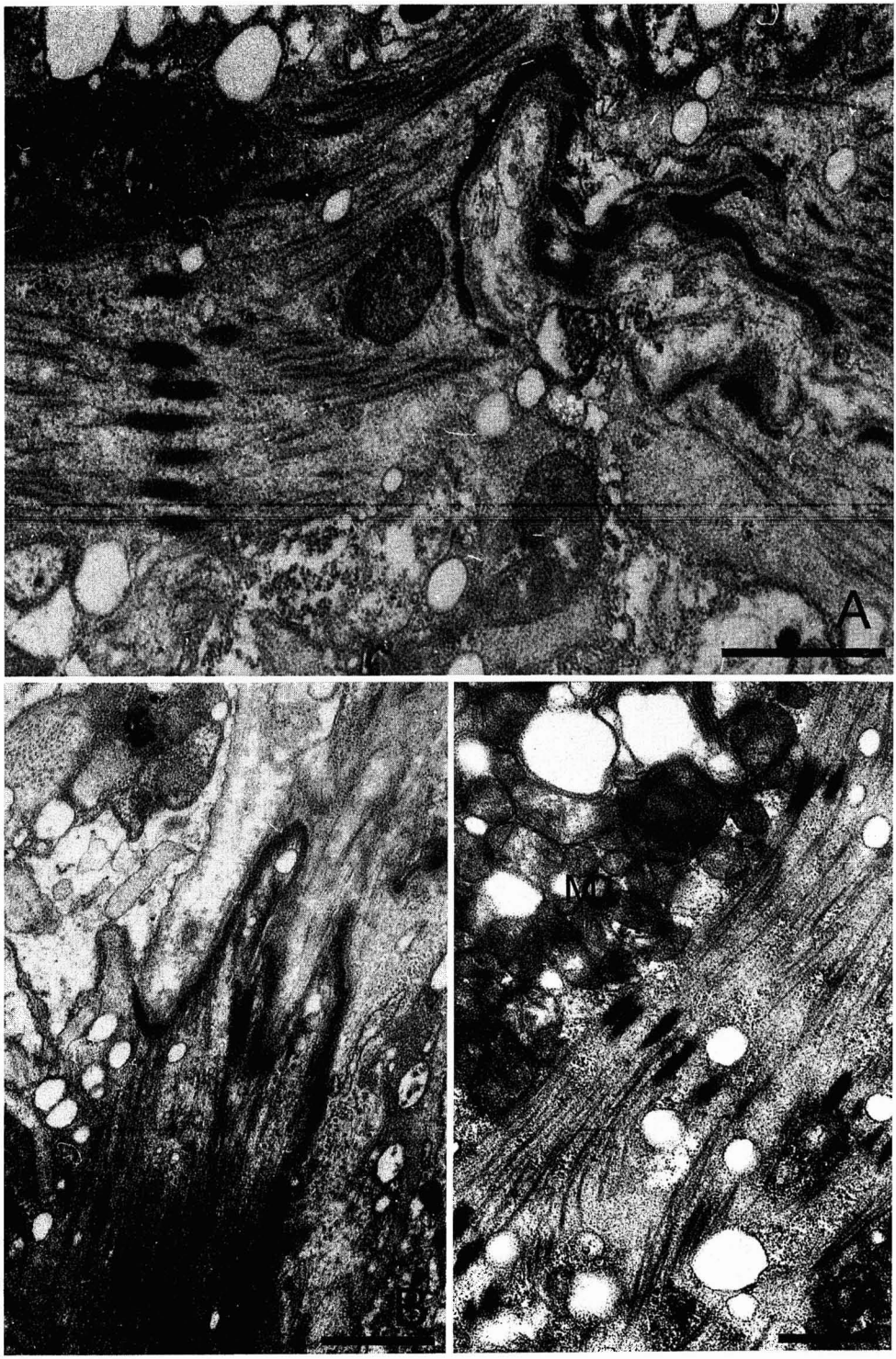

FIGURE 2. *Nautilus pompilius* myocardium. *A*, higher magnification of intercalated disk; *B*, longitudinal section of intercalated disk; *C*, open areas scattered throughout myofilaments and mitochondrial core of cell (M). Bar indicates 1 μm.

the top so that the diameter would appear to be small. Sarcoplasmic reticulum is clearly present in the muscles of the funnel that leads to the gills and the mantle cavity of *Nautilus* (Hochachka et al. 1978). We suggest, however, that neither their study nor ours unequivocally demonstrates a sarcoplasmic reticulum in the cardiac myofiber. More important to the present point, the density of the tubulelike structures is low relative to the density of sarcoplasmic reticulum in decapods (Dykens and Mangum 1979); even if they do prove to be components of a sacroplasmic reticular system, the system is not highly developed in the *Nautilus* heart.

While the sarcoplasmic reticulum is highly developed in all the decapods studied, the transverse tubule system either may (Jensen and Tjønneland 1977, Kawaguti 1963, Schipp and Schäfer 1969) or may not (Dykens and Mangum 1979) be present. There is no indication of a transverse tubule system in *Nautilus*.

Thus, the organization of the cardiac myofiber in *Nautilus* resembles the decapod myofiber in virtually no feature of relevance to the cardiovascular or metabolic performance of the system, and it does not appear to be specially adapted to sustain an exclusively aerobic and also highly mobile way of life. In addition, it is clear from the present results that the adaptations that permit contraction frequencies in excess of 250 beats/min (20°C) in squids occurred within the class Cephalopoda. Whether they are found only in the decapods or whether they also occur in the octopods is not known.

LITERATURE CITED

BOURNE, G. B., J. R. REDMOND, and K. JOHANSEN. 1978. Some aspects of hemodynamics in *Nautilus pompilius*. J. Exp. Zool. 205:63–70.

DYKENS, J. A., and C. P. MANGUM. 1979. The design of cardiac muscle and the mode of metabolism in molluscs. Comp. Biochem. Physiol. 62A:549–554.

HAYES R. L., and R. E. KELLY. 1969. Dense bodies of the contractile system of cardiac muscle in *Venus mercenaria*. J. Morph. 127:151–162.

HOCHACHKA, P. W., C. J. FRENCH, and J. MEREDITH. 1978. Metabolic and ultrastructural organization in *Nautilus* muscles. J. Exp. Zool. 205:51–62.

IRISAWA, N., A. IRISAWA, and N. SHIGETO. 1973. Physiological and morphological correlation of the functional syncytium in the bivalve myocardium. Comp. Biochem. Physiol. 44A:207–214.

JENSEN, H., and A. TJØNNELAND. 1977. Ultrastructure of the heart muscle cells of the cuttlefish *Rossia macrosoma* (Delle Chiaje) (Mollusca: Cephalopoda). Cell Tiss. Res. 185:147–158.

JOHANSEN, K., J. R. REDMOND, and G. B. BOURNE. 1978. Respiratory exchange and transport of oxygen in *Nautilus pompilius*. J. Exp. Zool. 205:27–36.

KAWAGUTI, S. 1963. Electron microscopy on the heart muscle of the cuttlefish. Biol. J. Okayama Univ. 9:27–40.

MOON, T. W., and W. C. HULBERT. 1975. The ultrastructure of the mantle musculature of the squid *Symplectoteuthis ovalaniensis*. Comp. Biochem. Physiol. 52B:145–149.

REDMOND, J. R., G. B. BOURNE, and K. JOHANSEN. 1978. Oxygen uptake by *Nautilus pompilius*. J. Exp. Zool. 205:45–50.

RUTHERFORD, J. G. 1972. The structure of the ventricle of *Elliptio complanatus*, a freshwater lamellibranch. J. Morph. 136:421–432.

SCHIPP, R., and A. SCHÄFER. 1969. Vergleichende elektronen mikroskopische Unterschungen an den zentralen Herzorganen von cephalopoden (*Sepia officinalis*). Zeit. Zellforsch. 98:576–598.